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Kinetic modelling of acrylamide formation during the finish-frying of french fries with variable maltose content

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23 **Abstract**

24 In light of a recent update in EU regulations governing levels of acrylamide in foodstuffs, further
25 understanding of the role of different precursors is fundamental to extending mitigation strategies
26 into a wider product range. Kinetic modelling was used to investigate the role of maltose in the
27 formation of acrylamide during the finish-frying of french fries. The maltose concentration of raw
28 white potato strips was systematically increased to observe the effect of this reducing disaccharide
29 on acrylamide formation. A mathematical model, incorporating glucose, fructose and maltose and
30 based on known Maillard reaction pathways, was developed which showed that acrylamide
31 formation from maltose only contributed <10% to the total acrylamide. An additional kinetic model
32 allowed for the formation of acrylamide directly from sugar-asparagine glycoconjugates. This
33 model suggested that under these conditions, it is unlikely that acrylamide is formed directly from
34 the maltose-asparagine conjugate.

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40 **Keywords:** potato fries, maltose, acrylamide, kinetic modelling, Maillard, glycoconjugates

41 **1. Introduction**

42 Since the discovery of high amounts of acrylamide, a probable carcinogen (IARC, 1994), in
43 processed foods, there has been a considerable effort to reduce the acrylamide content in processed
44 products either by manipulating the precursors and the processing conditions, or by obtaining raw
45 materials with low asparagine content (Halford, Curtis, Muttucumaru, Postles, Elmore, & Mottram,
46 2012). In light of new EU regulations (Commission Regulation (EU) 2017/2158) which came into
47 force in 2017 establishing tighter mitigation measures and benchmark levels for the reduction of
48 acrylamide in food, a concerted approach to acrylamide mitigation is required. Further
49 understanding of the role of different precursors under different conditions is fundamental to
50 extending mitigation strategies into a wider product range.

51 Maltose is an important disaccharide found in many foods, and is formed from the breakdown of
52 starch by β -amylases which are found in sweet potatoes, soya beans and germinating grains such as
53 barley and wheat. It is a reducing sugar and our hypothesis is that maltose will contribute to the
54 formation of acrylamide during high temperature processing of asparagine rich foods. Although the
55 breakdown pathways of disaccharides during the Maillard reaction are well documented
56 (Pischetsrieder & Severin, 1996; Smuda & Glomb, 2011), only a limited number of studies have
57 focused on the role of disaccharides and their corresponding Maillard-derived intermediates in the
58 formation of acrylamide (Koutsidis, De la Fuente, Dimitriou, Kakoulli, Wedzicha, & Mottram,
59 2008). In this study, we look at the relative contribution of maltose to acrylamide formation during
60 a typical food manufacturing process and compare the relative rates of formation from glucose,
61 fructose and maltose under different processing conditions. Combining established mechanisms for
62 acrylamide formation from monosaccharides and for the maltose-induced Maillard reaction, this
63 leads us to propose the following three possible pathways for maltose-induced acrylamide
64 formation : 1) breakdown of maltose via the Maillard reaction to provide a pool of reactive carbonyl
65 intermediates which subsequently react with asparagine to form acrylamide; 2) breakdown of

66 maltose to release glucose and subsequent formation of acrylamide from glucose and glucose
67 breakdown products; 3) direct breakdown of the maltose-asparagine glycoconjugate.

68 Multiresponse kinetic modelling is an effective technique to study the Maillard reaction
69 (Balagiannis, 2014; Parker, 2013; van Boekel, 2008). It can be used predictively to obtain
70 quantitative insight into complex reactions, but it can also serve as a means of probing and testing
71 different mechanistic pathways, thus providing strategies for controlling the reaction and, in this
72 case, minimising acrylamide. In particular, the kinetics of acrylamide formation has been studied in
73 simple mixtures of asparagine and reducing sugars, usually monosaccharides (De Vleeschouwer,
74 Van der Plancken, Van Loey, & Hendrickx, 2008a, 2008b, 2009a, 2009b; Knol, Linssen, & van
75 Boekel, 2010; Knol, Van Loon, Linssen, Ruck, van Boekel, & Voragen, 2005) under various
76 process conditions. Van der Fels-Klerx and coworkers (Nguyen, Van der Fels-Klerx, Peters, & van
77 Boekel, 2016; Nguyen, Van der Fels-Klerx, & van Boekel, 2017; Van Der Fels-Klerx et al., 2014)
78 extended this approach into biscuits, whilst Parker, Balagiannis, Higley, Smith, Wedzicha, &
79 Mottram (2012) used this approach in french fries taking into account the full complement of free
80 amino acids and reducing sugars in a complex food matrix. Parker et al. (2012) used a standard
81 protocol to manipulate the glucose and fructose levels in french fries, which were then processed in
82 a pilot-scale commercial fryer under different frying conditions. Uniquely, this model also
83 accounted for the moisture and heat transfer phenomena, as described for a one-dimensional infinite
84 slab by Ni and Datta (1999). Recently, this model was revisited, upgraded and extended to
85 approximate the mass and heat transfer events on a three dimensional basis (Balagiannis et al.,
86 2016).

87 In this study, the same process was used in order to investigate the role of maltose in acrylamide
88 formation during the finish frying of white potato fries. Although white potatoes are not a natural
89 source of maltose, they were selected for this study because the matrix can serve as a versatile
90 substrate in which to manipulate and study the effects of maltose content on the kinetics of
91 acrylamide formation. One further advantage was the fact that the behaviour of glucose and

fructose, and the heat and moisture transfer phenomena, have already been determined in white potato fries (Parker et al., 2012), providing an ideal starting point for studying the relative contribution of maltose, in comparison to glucose and fructose.

The aims of this study were to use multi response kinetic modeling to understand the role of maltose in acrylamide formation in a real product, using French fries with variable maltose content prepared under pilot-scale manufacturing conditions. By incorporation of similar data using glucose and fructose from Parker et al. (2012), we developed one model that can explain and compare the relative contribution from each of the sugars during acrylamide formation. Further to the role of maltose, we also take the opportunity provided by this rich data set to develop a better understanding of the mechanism of acrylamide formation by considering both the specific amino acid pathway where reducing sugars react directly with asparagine to form acrylamide, and a generic amino acid pathway where reactive precursors are formed via the Maillard reaction.

2. Materials and methods

2.1 Materials

Ranger Russet potatoes were obtained from the 2013 crop and stored at 7-9 °C until use. All chemicals were supplied as described by Parker et al. (2012).

2.2 Preparation of french fries with variable maltose content

Ranger Russet potatoes were treated and processed as described by Parker et al. (2012) except that the glucose and fructose dips were replaced by a maltose dip. The commercial production of french fries involves a multi-step process which was reproduced as closely as possible on a pilot scale. Briefly, the raw potatoes from harvest or storage were washed, peeled, preheated (54 °C, 30 min) and cut into strips. The cross section of the raw potato strips was 7.5×7.5 mm and the length varied between 7.5 and 12.5 cm. Subsequent blanching at 80 °C for 10 min causes some leaching of sugars and was followed by a dipping step where the potato strips were immersed into a maltose solution of varying concentration (70-75 °C, 30 s). The loss of sugars during blanching, and the requirement to restore their concentration, is a part of the process that allows the control of the Maillard reaction,

118 thus influencing colour and acrylamide formation. The blanched potato strips were dipped in
119 maltose solutions of the following concentrations: 0% (only water), 1, 2, 3, 4 and 14% (w/v). The
120 outlying value of 14% was chosen to achieve a final maltose content similar to that of blanched
121 sweet potato strips. In sweet potatoes, the maltose increases up to ~2% during the manufacturing
122 process, depending on the variety and the processing conditions (Takahata, Noda , & Nagata, 1994).
123 Application of a 14% dip to white potato strips took the maltose content up to 1.4% (wet weight) in
124 the blanched strip. During blanching and dipping there was an uptake of ~10% of water which was
125 removed by a subsequent drying step (60 °C, 10 min). The potato strips were then par-fried (190 °C,
126 50 s) in canola oil, packaged and frozen (-20 °C) before finish-frying in canola oil. Typically finish-
127 frying is carried out for 2-5 min, to an end point based on the desirable colour, texture, and flavour.
128 Each batch (~220 g) was finish-fried at 165 °C for 10 different time points (0.5, 1.0, 1.5, 2.0, 2.5,
129 3.0, 3.5, 4.0, 4.5 and 5.0 min). Immediately after frying, the samples were submerged in liquid
130 nitrogen to prevent any further reaction, and stored at -20 °C until further analysis. A similar
131 process was followed for a second batch of potatoes that was dipped in a solution of 2% maltose
132 and then fried at 175 and 185 °C.

133 **2.3 Chemical analysis**

134 Acrylamide concentration was determined using the method described by Parker et al. (2012) using
135 an SPE clean-up procedure, analysis by LC-MS/MS and quantification using ¹³C₃-labeled
136 acrylamide as an internal standard.

137 The analysis of sugars was performed by HPLC according to the method reported by Parker et al.
138 (2012). The concentration of glucose, fructose, maltose, and sucrose in samples was calculated via
139 external calibration with authentic standards.

140 Free amino acids were measured via derivatisation as described by Parker et al. (2012). In
141 summary, the free amino acids Asn, Gln, Arg, Ala, Gla, Val, Asp, Ser, Lys, Phe, Tyr, Thr, Ile, Met,
142 Pro, His, Leu, and Gly were derivatised by *o*-phthalaldehyde and 9-fluorenylmethoxycarbonyl
143 chloride in an autosampler immediately before injection in an Agilent HPLC 1100 series.

144 Analysis of water and total fat content were performed as described by Parker et al. (2012). Briefly,
145 moisture was determined by oven drying 2 g of sample until constant weight in a fan oven at 125
146 °C. Fat was extracted, hydrolysed and methylated to produce fatty acid methyl esters which were
147 quantitated by GC-MS.

148 Colour formation was assessed with an Agtron E-30 Analyzer (Agtron Inc., Reno, NV) as described
149 in Parker et al. (2012).

150 **2.4 Kinetic modelling**

151 Multiresponse modelling and model simulations were performed using the Athena Visual Studio
152 software package (Athena Visual Software Inc., Naperville, IL).

153 **3. Results and discussion**

154 **3.1 General observations**

155 The maltose, glucose, fructose, sucrose, free amino acids and acrylamide contents were measured in
156 order to monitor the acrylamide formation in relation to the initial concentration of Maillard
157 reaction precursors. The data are all displayed graphically in supplementary Figure S1. The
158 concentration of sucrose did not alter during the course of the reaction (results not shown). The
159 temperatures that are typically applied during frying far exceed 100 °C, which results in a
160 continuous evaporation and loss of moisture. Furthermore, the fried strips absorb oil during frying:
161 70-80% of the oil that is detected in the final finish-fried product adheres to the surface of the fry,
162 while the other 20-30% is oil that is trapped in the crust (Aguilera & Gloria-Hernandez, 2000). The
163 experimental data confirmed that the longer the frying time, the greater the loss of moisture and the
164 higher the amount of oil that was absorbed. In order to compare the trend of the components of
165 interest with time, it was necessary to normalise their concentration by removing any effect
166 introduced by their moisture and the fat content. Thus, throughout this paper, the concentration of
167 the analytes is reported on a defatted dry weight basis.

168 The experiment was performed with two different batches of white potatoes. In the first batch, the
169 concentration of maltose dip ranged from 0-14% and the fries were processed at 165 °C (samples

M0, M1, M2, M3, M4 and M14, Figure 1). In samples M0, M1, M2 and M3, all precursors decreased over time, and there was a continuous increase in acrylamide. However, in samples M4 and M14, i.e. the two samples with the highest concentration of maltose, glucose either remained stable (M4) or there was a small increase with time (M14). This is probably due to the formation of glucose from the degradation of maltose, which in the samples with high maltose may occur faster than the reaction of glucose with amino groups, thus the net result is a small accumulation of glucose. The second batch of white potatoes was dipped in a 2% maltose solution and then samples were finish-fried at either 175 °C (M2'175) or 185 °C (M2'185). These two potato batches had different concentrations of precursors at the start: the raw fries in the first batch had on average 12.7 mmol/kg dry weight glucose and 131 mmol/kg dry weight of total free amino acids, whilst the raw fries in the second batch had half as much glucose (6.1 mmol/kg dry weight) but almost double total free amino acids (238 mmol/kg dry weight). These differences, whilst not immediately comparable with the previous batch, provide additional information for the kinetic modelling and strengthen the model.

Even though the maltose dip levels varied from 0% to 14%, the range of acrylamide formation was narrow. Sample M0 reached a maximum of 5.4 µmol/kg dry weight, as a result of the reaction of the endogenous fructose and glucose present in the potato strips. Sample M1 had a maximum of 13.4 µmol/kg dry weight, while the other samples (i.e. M2, M3, M4 and M14) had similar final acrylamide concentrations in the range 20-24 µmol/kg dry weight. Beyond the 2% dip, further increases in maltose concentration seem to have little impact on acrylamide formation, and this was not due to exhaustion of the asparagine. In a similar experiment, when the glucose and fructose dips varied up to just 2%, the final concentration of acrylamide was much higher, ranging from 8.2–86 µmol/kg dry weight (Parker et al., 2012). It is striking that the amount of acrylamide formed in the batches with 2, 3, 4 and 14% maltose dip concentrations was similar even though it corresponded to different degrees of maltose consumption (Table 1). For example, the fries with 2% and 14% maltose dip showed similar acrylamide formation (20.1 and 20.8 µmol/kg dry weight respectively),

196 which corresponded to different losses of maltose (10.7 and 52.4 $\mu\text{mol/kg}$ dry weight respectively).
197 Similarly, the study of the above systems in relation to colour formation (Table 1) showed that the
198 fries with added maltose developed less colour than the systems with added glucose or fructose. In
199 fact, after 5 min of frying, the samples which had been dipped in 14% maltose and 2% fructose had
200 a similar colour, but M14 has consumed twice as much sugar but formed a fraction of the
201 acrylamide.

202 It is likely that the maltose is converted into colourless higher molecular weight compounds. Frank
203 and Hofmann (2000) have shown that colour formation in maltose is hindered by the 1,4-glycosidic
204 link which is unhydrolysable under typical food preparation conditions. The maltose-derived
205 glycosides are colourless compared to their monosaccharide counterparts which undergo further
206 eliminations to generate coloured aglycones. Maltose also forms anhydrosugars, furosine and
207 unreactive maltose specific compounds as suggested by Hollnagel and Kroh (2000) or other
208 heterocycles with the 1-4 glycosidic link still intact, as shown by Kramhoeller, Pischetsrieder, &
209 Severin (1993). Hollnager and Kroh (2000) report that the major intermediate formed from maltose
210 degradation in a dry system at 100 °C was 1,4-dideoxyhexosulose (1,4DDH) and this built up to
211 concentrations of 18 mol% in relation to maltose. It is reactive in terms of condensations reactions,
212 colour formation and the formation of heterocycles. However, they reported almost insignificant
213 amounts of C2/ C3 α -dicarbonyls and α -hydroxycarbonyls, which may explain the formation of
214 relatively little acrylamide. No-one as yet has shown the reactivity of 1,4DDH (an α -dicarbonyl)
215 towards acrylamide formation. It seems that in french fries, the addition of maltose by dipping from
216 the level of 2% onwards does not affect significantly the formation of acrylamide, at least not as
217 much as it is affected by the addition of different levels of glucose or fructose shown by Parker et
218 al., (2012).

219 To conclude, during frying of potato strips, maltose was less effective than glucose and fructose, in
220 relation to the formation of Maillard products and acrylamide. This is in agreement with the fact
221 that in Maillard browning, disaccharides are less reactive than monosaccharides (Lingnert, 1990;

222 Wedzicha & Kedward, 1995). In addition, Koutsidis et al. (2008) reported that maltose was less
223 potent than glucose and fructose with respect to acrylamide formation in a low-moisture waxy
224 maize starch model system that was heated at 160 °C.

225 **3.2 Developing the kinetic mechanism**

226 Given the complexity of the reactions involved, one approach to gaining a better understanding of
227 these two pathways is to mathematically model the process based on the known chemistry of the
228 reactions. Modelling a complex reaction such as acrylamide formation is a challenging task. An
229 effective approach is to identify the few rate limiting steps that regulate the kinetic behaviour of the
230 reaction and develop a mathematical model based on them. As a result, the dense network of sub-
231 reactions that constitute a complex reaction are reduced to a limited number of kinetically important
232 steps. Each kinetic step incorporates several chemical pathways on the basis that the rate of a group
233 of reactions is determined by the speed of the slowest step. This approach has been applied
234 successfully on several occasions in the field of Maillard chemistry and acrylamide formation.
235 (Balagiannis et al., 2016; Balagiannis, Parker, Pyle, Desforges, Wedzicha, & Mottram, 2009;
236 Brands & van Boekel, 2002; De Vleeschouwer et al., 2008b; Knol et al., 2005; Low, 2006; Martins
237 & van Boekel, 2004; Parker et al., (2012); Wedzicha, Mottram, Elmore, Koutsidis, & Dodson,
238 2005). In earlier work on monosaccharides, we initially expressed the complex chemistry of
239 acrylamide formation in relation to glucose and fructose in a few kinetic steps which included both
240 the generic and specific amino acid pathways. However, there was high uncertainty in the kinetic
241 parameters, particularly those driving the split between the generic and specific amino acid
242 pathway, so a simpler model was published where these two pathways were collapsed into one,
243 incorporating two kinetically important intermediates (Parker et al., 2012). Subsequent iterations of
244 the model took into account the fact that more acrylamide is likely to be formed at the edges and
245 corners of the fries, as is observed anecdotally by the greater colour formation at the edges and
246 corners of the fries (Balagiannis et al., 2016).

For the purpose of the present study, the previously published kinetic mechanism (Balagiannis et al., 2016) was the starting point for development of a new model incorporating maltose. Several modelling trials indicated that the estimates could be improved if the transition from glucose to a group of intermediates (Int2) was expressed by just one kinetic step instead of two. Furthermore, it is well documented that Maillard reaction intermediates that contain an intact sugar backbone fragment via retroaldolisation and oxidative fission reactions (Nursten, 2005) producing α -dicarbonyls, α -hydroxycarbonyls or acids from the reducing terminus of the sugar, and tetroses and trioses from the other end. These highly reactive compounds readily react with free amino acids. This molar relationship was included in the updated kinetic mechanism and was expressed by parameter p which reflects the fact that the number of moles of Int2 formed may be greater than the number of moles of glucose lost. The new mechanism accounted for a larger consumption of free amino acids during the reaction of Int2 to form acrylamide and Maillard products, removing the necessity for the reaction previously associated with k_{aa} which had been included in previous models to account for the undefined loss of free amino acids (Balagiannis et al., 2016; Parker et al., 2012).

3.3 Incorporation of maltose into the kinetic model

The kinetic mechanism was then expanded to include the chemistry and the kinetics of acrylamide formation in relation to maltose. This was achieved by taking into account the study by Mundt and Wedzicha (2005). These authors modelled the formation of melanoidins in a solution of 0.2 M sodium acetate/glacial acid buffer (pH 5.5), containing equimolar amounts of maltose (0.25 M) and glycine, which was heated at 70 °C for several time points. They followed a radiochemical approach to propose a kinetic mechanism which described how maltose participates in the Maillard reaction. In summary, they suggested that maltose reacts with glycine to form an intermediate which breaks down to glucose and a second reactive intermediate, identified by Hollnager et al. (2000) as 1,4 DDH. The latter reacts further to form melanoidins via Int2. Hence, we propose Kinetic Mechanism 1 (Figure 2) which shows how maltose, glucose and fructose participate in the formation of

acrylamide. In Kinetic Mechanism 1 the role of maltose is dual: on the one hand, maltose itself (as a reducing sugar) participates in the Maillard reaction and acrylamide formation, through a three-step process and the formation of two pools of intermediates, Int1_{mal} and Int (Pathway 1). On the other hand, one glucose molecule is released from Int1_{mal} through a “peel off” mechanism, (Mundt and Wedzicha, 2005) and maltose contributes to the formation of acrylamide via the glucose and fructose (Pathway 2). The following differential equations are derived from Kinetic Mechanism 1 (Figure 2) and comprise Model 1.

$$\frac{d[\text{Glu}]}{dt} = -k_1[\text{Glu}][\text{FAA}] + k_{2\text{mal}}[\text{Int1}_{\text{mal}}] - k_8[\text{Glu}]$$

$$\frac{d[\text{Fru}]}{dt} = -k_6[\text{Fru}] + k_8[\text{Glu}]$$

$$\frac{d[\text{Mal}]}{dt} = -k_{1\text{mal}}[\text{Mal}][\text{FAA}]$$

$$\frac{d[\text{FAAs}]}{dt} = -k_3[\text{Int2}][\text{FAA}]$$

$$\frac{d[\text{Asn}]}{dt} = -k_3[\text{Int2}][\text{Asn}]$$

$$\frac{d[\text{Acr}]}{dt} = k_3[\text{Int2}][\text{Asn}] * F_{\text{Asn}} * \text{Asn}$$

$$\frac{d[\text{Int1}_{\text{mal}}]}{dt} = k_{1\text{mal}}[\text{Mal}][\text{FAA}] - k_{2\text{mal}}[\text{Int1}_{\text{mal}}]$$

$$\frac{d[\text{Int2}]}{dt} = (1 + p)(k_1[\text{Glu}][\text{FAA}] + k_6[\text{Fru}] + k_{2\text{mal}}[\text{Int1}_{\text{mal}}]) - k_3[\text{Int2}][\text{FAA}]$$

where F_{Asn} is the fraction of asparagine that is converted to acrylamide. Note that during the reactions associated with k_1 and $k_{1\text{mal}}$, the free amino acids are regenerated so there is no loss of free amino acids associated with these pathways.

Athena Visual Studio was used to solve the simultaneous differential equations and estimate the parameters using the data from the maltose-dipped fries, combined with the data from the fries with variable glucose and fructose content taken from Parker et al. (2012). The parameter estimates are shown in Figure 2. Overall, the parameters were good with respect to their 95% confidence

intervals. The fit of the model to the experimental values is very good, and for the maltose-dipped samples this is demonstrated in Figure 1. The overall quality of the fit is shown in Figure 3, where the observed vs. predicted plots include all the measured responses from all available datasets. In all cases linear regression gave slopes close to 1.0 (0.96 to 0.103) and generally the R^2 was > 0.95 . The exceptions were for the free amino acids where there was more scatter, but the slope was still 1.0. The estimate for parameter F_{Asn} is 32×10^{-4} , which implies that 0.3% of the asparagine is converted to acrylamide. Also, parameter p was estimated as 0.85, which implies that from 1 mole of intermediates with an intact sugar backbone, 1.85 moles of fragmentation products are formed. Comparison of the kinetics of the formation of acrylamide from the three sugars was carried out in Athena using model simulations. Using the rate constants from Figure 2, the model was run at 165 °C where the concentration of the total FAA and Asn were set to 164 and 60 mmol/kg dry weight respectively (the same as in M0), and the initial acrylamide (generated during par-frying) was set to zero. In turn, each of the sugars was set to 40 mmol/kg dry weight (falling within the experimental ranges used) whilst the other two were set to zero, in order to determine the individual contribution from each of the sugars. The simulations are shown in Figure 4a, and it is clear that maltose makes only a small contribution to the total acrylamide formation. Figure 4b shows the data as a % of total, where it is clear that fructose is initially faster than glucose under these particular conditions, and the contribution from maltose increases with frying time to a maximum of 10% of the total. However for the first 200 seconds, the contribution from maltose is less than 5%.

3.4 Incorporation of specific and generic pathways into the kinetic model

During food processing, two related pathways have been proposed for acrylamide formation (Parker et al., 2012): the “specific amino acid pathway” and the “generic amino acid pathway”. Whereas Kinetic Mechanism 1 provides a very good model to describe the formation of acrylamide during frying, it does not discriminate between the generic and the specific amino acid pathways. The generic amino acid pathway involves the Maillard reaction (top line, Figure 5) - the reaction between any of the free amino acids present in the food with reducing sugars to form a series of

Schiff bases. These rearrange to form the corresponding Amadori rearrangement products, and the subsequent cascade of reactions results in the formation of highly reactive deoxyosones and short chain carbonyl intermediates, particularly α -dicarbonyls such as glyoxal and methylglyoxal, α -hydroxycarbonyls such as glycolaldehyde and hydroxypropanone, and also trioses and tetroses. Many of these carbonyls react with amino acids more effectively than glucose does (Hofmann, 1999) and in particular with free asparagine to form acrylamide. This could be via the α -hydroxycarbonyls as detailed by Stadler, Robert, Riediker, Varga, Davidek, Devaud et al. (2004) (centre of Figure 5), or via α -dicarbonyls as shown by Koutsidis et al. (2008) and Amrein, Limacher, Conde-Petit, Amado, & Escher (2006) (right-hand side of Figure 5). The chemistry of this latter pathway has been less well defined but is certainly different when there is a vicinal dicarbonyl involved. It may proceed via the 3-aminopropionamide, returning a dicarbonyl, or via β -elimination (Stadler et al., 2004). In both cases, whether the reactive species is a dicarbonyl or a hydroxycarbonyl, the Maillard reaction provides a source of those particularly reactive species that asparagine requires in order to induce acrylamide formation. These pathways are certainly Maillard assisted.

During the specific amino acid pathway, asparagine reacts directly with the reducing sugars present in the food to form a sugar-asparagine glycoconjugate which dehydrates to a Schiff base (left-hand side of Figure 5). This is followed by a series of reactions which involve the decarboxylation of the Schiff base (thus by-passing the formation of the Amadori rearrangement product), and a subsequent tautomerism which leads to the formation of acrylamide (Stadler et al., 2004). The chemical mechanism involved in this pathway is the same as for the Maillard assisted pathway via hydroxycarbonyls (Figure 5). However, the kinetics of this pathway are different, because it does not require the initial formation of a source of reactive carbonyls. For the remainder of the kinetic discussion, we will refer to this pathway as the specific sugar-Asn pathway.

It is important at this stage to highlight the difference between a chemical mechanism and a kinetic mechanism. Chemically, what we have referred to previously as the specific amino acid mechanism

includes the reaction of asparagine directly with C6 reducing sugars and other α -hydroxycarbonyls which are formed during the Maillard reaction (Stadler et al., 2004). However kinetically, the specific reaction with Maillard-derived α -hydroxycarbonyls is effectively initiated by any amino acid, and is therefore indistinguishable, from a kinetic point of view, from the generic amino acid pathway. This Maillard-assisted specific amino acid pathway follows the chemistry of the specific sugar-Asn pathway, but the kinetics of the general pathway.

Although the generic amino acid pathway involves several more steps, the reaction of asparagine with the highly reactive carbonyl intermediates may be faster than the first step of the specific sugar-Asn pathway. The proportion that each of these pathways contributes to the formation of acrylamide in food is not yet clear, and is likely to depend on the composition of the raw material, as discussed by Ngyuen et al. (2016).

Taking this into consideration, a new kinetic mechanism was proposed (Kinetic Mechanism 2, Figure 1). The right-hand side of the mechanism expresses the specific sugar-Asn pathway, where each sugar reacts with asparagine, and acrylamide is formed via a single kinetically important step. The left-hand side of the mechanism expresses the generic amino acid pathway where acrylamide is formed from the reaction of asparagine with a group of very reactive intermediates *Int*₂, as was described in detail previously in this paper. Note that this also includes the reaction of reactive hydroxycarbonyls via the Stadler chemical mechanism. The differential equations derived from Kinetic Mechanism 2 (Model 2) are:

$$\frac{d[\text{Glu}]}{dt} = -k_1[\text{Glu}][\text{FAA}] + k_{2\text{mal}}[\text{Int1}_{\text{mal}}] - k_8[\text{Glu}] - k_g[\text{Glu}][\text{Asn}]$$

$$\frac{d[\text{Fru}]}{dt} = -k_6[\text{Fru}] + k_8[\text{Glu}] - k_f[\text{Fru}][\text{Asn}]$$

$$\frac{d[\text{Mal}]}{dt} = -k_{1\text{mal}}[\text{Mal}][\text{FAA}] - k_m[\text{Mal}][\text{Asn}]$$

$$\frac{d[\text{FAAs}]}{dt} = -k_3[\text{Int2}][\text{FAA}] - k_g[\text{Glu}][\text{Asn}] - k_f[\text{Fru}][\text{Asn}] - k_m[\text{Mal}][\text{Asn}]$$

$$\frac{d[\text{Asn}]}{dt} = -k_3[\text{Int2}][\text{Asn}] - k_g[\text{Glu}][\text{Asn}] - k_f[\text{Fru}][\text{Asn}] - k_m[\text{Mal}][\text{Asn}]$$

$$\frac{d[\text{Acr}]}{dt} = k_3[\text{Int2}][\text{Asn}] * F_{\text{Asn}} + k_g[\text{Glu}][\text{Asn}] + k_f[\text{Fru}][\text{Asn}] + k_m[\text{Mal}][\text{Asn}]$$

$$\frac{d[\text{Int1}_{\text{mal}}]}{dt} = k_{1\text{mal}}[\text{Mal}][\text{FAA}] - k_{2\text{mal}}[\text{Int1}_{\text{mal}}]$$

$$\frac{d[\text{Int2}]}{dt} = (1 + p)(k_1[\text{Glu}][\text{FAA}] + k_6[\text{Fru}] + k_{2\text{mal}}[\text{Int1}_{\text{mal}}]) - k_3[\text{Int2}][\text{FAA}]$$

This model contains three more parameters than the model 1. Athena Visual Studio was used to solve the simultaneous differential equations and estimate the parameters. As for Model 1, the combined data were used for parameter estimation giving rise to Model 2. The estimated parameters of Model 2, as calculated by Athena Visual Studio, are shown in Figure 2. The fit of the model to the experimental data is very good and similar to the fit of the model of Kinetic Mechanism 1. The fits are shown in supplementary figures S2 and S3. Similar to Model 1, parameter p was estimated as 0.66, which implies that from 1 mole of intermediates with an intact sugar backbone, 1.66 moles of fragmentation products are formed. All the estimates for the reaction rate constants of Kinetic Mechanism 2 were estimated with acceptable 95% confidence intervals (Figure 2) with the exception of k_m which was returned as lower bound (zero). This shows that both generic and specific sugar-Asn pathways are likely to contribute to the formation of acrylamide in french fries and model simulations suggest that there is a significant contribution from both. Acrylamide is formed via both routes when the participating sugars are glucose and fructose, but the formation from maltose via the maltose glycoconjugate is kinetically insignificant and maltose contributes to the generation of acrylamide only via the generic amino acid pathway or via glucose.

3.5 Discussion of the chemical mechanisms

We can draw some significant conclusions from the kinetic modelling. The data obtained could not be fitted to the specific sugar-Asn route, but good models were obtained when the model was based on the generic amino acid pathway (Model 1) and when we allowed the model to incorporate the specific sugar-Asn route as well (Model 2). Although in modelling terms, Model 1 is preferable because it is simpler, Model 2 shows that both routes are likely to contribute. To our knowledge, this is the first kinetic model of acrylamide formation which has successfully incorporated both the

specific sugar-Asn and the generic amino acid pathways. Early models tended to favour the specific sugar-Asn pathway as proposed by Stadler et al. (2004), and confirmed by Zyzak et al. (2003) who showed evidence of the intermediate decarboxylated Amadori product in a high sugar system. Knoll and coworkers modelled their data from aqueous buffered asparagine/glucose (Knol et al., 2005) or asparagine/fructose (Knol et al., 2010) model systems using a two-step reaction based on the specific amino acid pathway. This was successfully extended into low moisture systems by De Vleeschouwer and co-workers (2008b, 2009b) who found that the best kinetic model fitted the specific sugar-Asn pathway for acrylamide formation, and also included the Maillard reaction as a browning pathway, but not as a source of reactive intermediates for acrylamide formation. This fit is not unexpected from a system that contains just one amino acid and no other source of reactive carbonyl species. However, this model also fitted when the data were collected from a potato-based matrix (De Vleeschouwer et al., 2008a). Van der Fels-Klerx and co-workers (Nguyen et al., 2016, 2017) considered both the generic and specific sugar-Asn pathways when they developed their model for acrylamide formation in biscuits, and again found the better models involved just asparagine, although in their latest model, (Van Der Fels-Klerx et al., 2014) which incorporated the participation of other free amino acids, the best model included the Maillard reaction as a sink for the excess sugars, but not as a source of reactive intermediates for acrylamide formation. Wedzicha et al. (2005) and Low, Mottram, & Elmore (2006) proposed a general amino acid pathway for the formation of acrylamide and developed a kinetic model for the formation of acrylamide in low sugar systems (potato, rye and wheat products). They observed the competition between the formation of acrylamide and other Maillard-derived compounds, from a common pool of intermediates, providing evidence for a significant contribution from the generic amino acid pathway. In our earlier work (Parker et al., 2012), we initially considered both specific and general amino acid pathways, but the model with the best fit was based on a three-step reaction relying on the Maillard reaction to generate highly reactive intermediates (generic amino acid pathway) and there were insufficient data to split the model into a generic and a specific component. In summary,

there is good evidence for both the existence of the specific sugar-Asn pathway in model systems and biscuits, and the existence of the general amino acid pathway in cereals and potatoes. In this paper, we have shown that these routes can co-exist during the frying of potato fries, and it is likely that the contribution from the Maillard reaction depends certainly on the composition of the food, and possibly on other factors. At one extreme, the glucose/asparagine model systems of Knoll and co-workers (2010, 2005) were dominated by the specific pathway, since none of the particularly reactive amino acids was present to promote the Maillard reaction and the formation of reactive carbonyls. In the high sugar biscuit models (Nguyen et al., 2016, 2017) the molar ratio of reducing sugars to asparagine was high (~50:1), and the contribution from the Maillard-assisted route may be small enough that the best fit model is still based on the specific sugar-Asn pathway. However in french fries, the molar ratio of reducing sugars to asparagine was low (~1:10), and model 2 suggests a much greater contribution from the Maillard reaction. Under these conditions, the formation of acrylamide is “Maillard assisted” by the accumulation of reactive carbonyl intermediates. This is an important point to emphasise – the fact that the chemical mechanisms can change depending on the composition of the food.

4. Conclusion

This is the first time that a holistic and robust model of acrylamide formation has been developed in a real and complex food incorporating glucose, fructose and maltose, as well as moisture and heat transfer parameters, and giving some insight into the relative contribution from different formation pathways. At equimolar concentrations, maltose contributed < 10% to the total acrylamide formation, giving us less concern over acrylamide levels in foods containing or generating maltose (and by extension, higher oligomers of glucose). The first model demonstrates that the breakdown of maltose and subsequent formation of acrylamide is relatively slow, whether this be via a maltose Maillard intermediate route or via the “peel off” of glucose. The pathways from maltose require three rate limiting steps as opposed to two from glucose or fructose. Model 2 which allowed for the formation of acrylamide directly from sugar-asparagine glycoconjugates, showed there was a

448 contribution from the glucose and fructose glycoconjugates, but not from maltose glycoconjugate.
449 The more we understand about the formation pathways of acrylamide in real food systems, the
450 better we are equipped to develop appropriate mitigation strategies.

451 **Supporting information**

452 **Figure S1.** Graphs showing fit of all experimental data to model 1, generated from Kinetic
453 Mechanism 1.

454 **Figure S2.** Graphs showing fit of all experimental data to model 2, generated from Kinetic
455 Mechanism 2.

456 **Figure S2.** Graphs showing predicted vs. observed values for model 2, generated from Kinetic
457 Mechanism 2.

458 **5. References**

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Table 1. Comparison of the rate of change of maltose and acrylamide in the maltose-dipped french fries and the 2% glucose-or fructose-dipped fries (data taken from Parker et al. (2012)) after finish frying at 165 °C for 5 min.

Addition level	Maltose						Glucose	Fructose
	0%	1%	2%	3%	4%	14%	2%	2%
Sug_{ini}^a	-	16.4	24.2	41.6	47.3	164	63.0	72.8
Sug_{fin}^b	-	7.1	13.5	20.7	26.3	111	27.3	50.4
Sug_{ini}-Sug_{fin}^c	-	9.2	10.7	20.9	21.0	52.4	35.8	22.4
Acr_{fin}-Acr_{ini}^d	4.5	10.9	20.1	17.5	19.4	20.8	72.7	75.4
Colour (Agtron Score)^e	70.8	63.2	56.1	52.9	53.4	34.6	26.3	34.8

^aconcentration of the added sugar in fries before finish-frying (mmol/kg dry weight),

^bconcentration of the added sugar in fries after finish-frying (mmol/kg dry weight), ^closs of added sugar (mmol/kg dry weight) during finish frying, ^dconcentration of acrylamide in fries after finish frying (µmol/kg dry weight), ^ecolour after finish frying

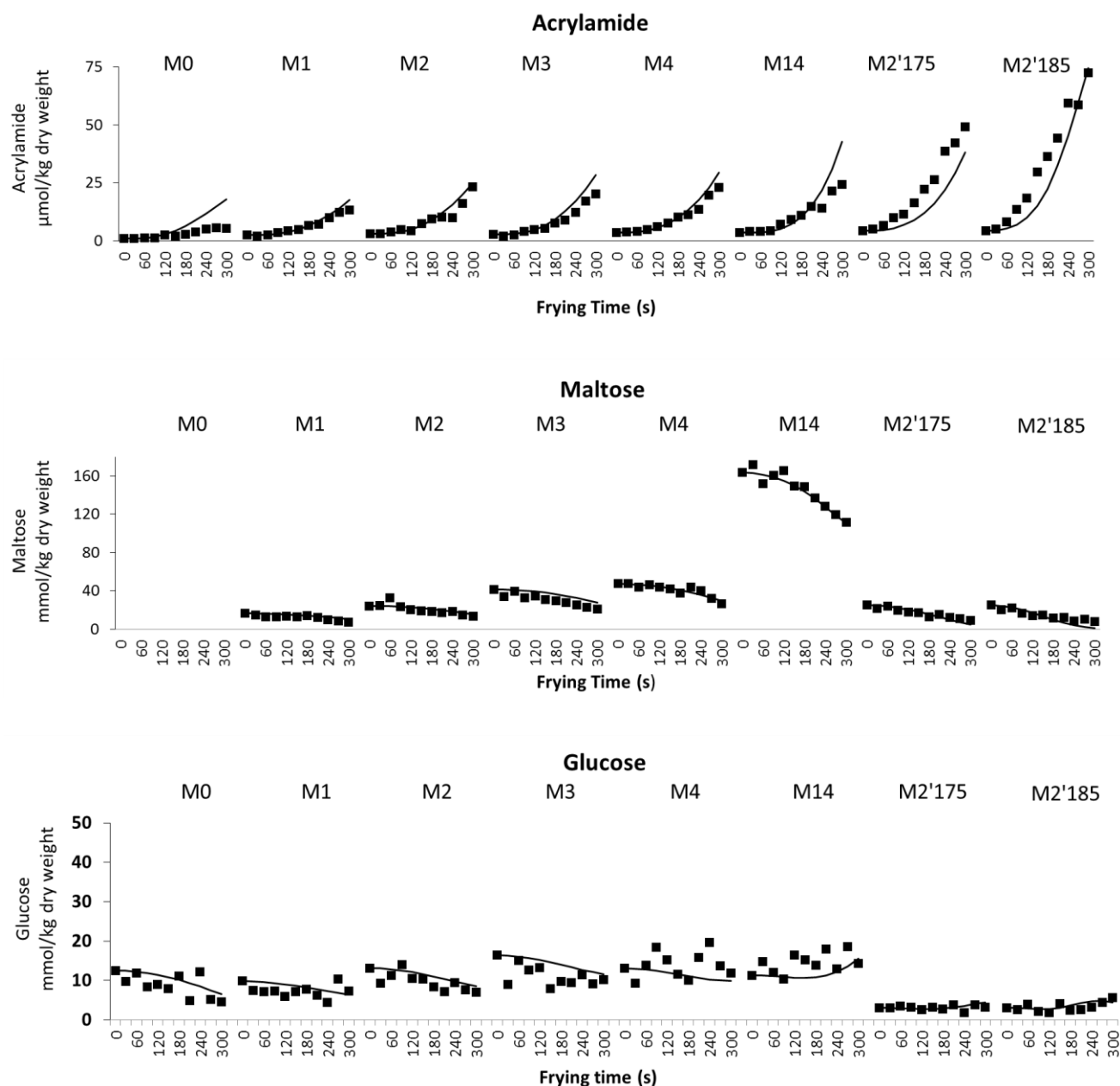
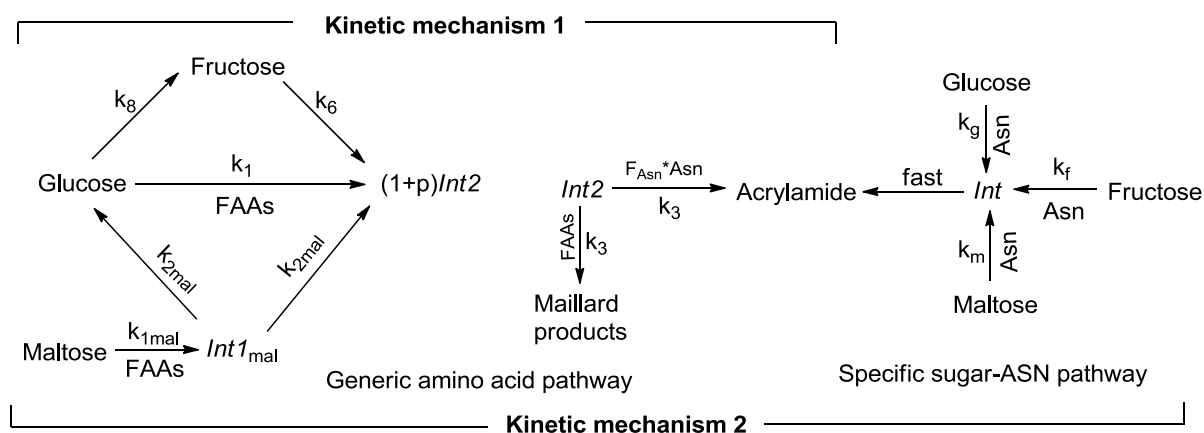


Figure 1. Acrylamide, maltose and glucose concentrations as a function of time (0–300 s) during finish-frying of eight batches of maltose-dipped potato strips at 165 °C (M0, M1, M2, M3, M4 and M14 represent 0, 1, 2, 3, 4 and 14% dip respectively), 175 °C (M2'175 = 2% dip) and 185 °C (M2'185= 2% dip). Symbols (■) are the experimental data points and the line (—) shows kinetic Model 1 derived from the combined data set using Kinetic Mechanism 1 (Figure 2).



Parameter	Model 1 based on Kinetic Mechanism 1		Model 2 based on Kinetic Mechanism 2	
	Optimal estimate $\times 10^4$	95% Confidence interval $\times 10^4$	Optimal estimate $\times 10^4$	95% Confidence interval $\times 10^4$
k_1 ($\text{mmol}^{-1} \text{ kg s}^{-1}$)	2.2	± 0.03 (1%)	2.1	± 0.3 (16%)
k_3 ($\text{mmol}^{-1} \text{ kg s}^{-1}$)	72	± 25 (35%)	220	± 62 (28%)
k_6 (s^{-1})	165	± 9 (5 %)	161	± 13 (8%)
k_8 (s^{-1})	68	± 8 (12%)	73	± 15 (20%)
p	8550	± 2510 (29%)	6640	± 2713 (41%)
F_{Asn}	32	± 4 (14%)	18	± 7 (38%)
k_{1mal} ($\text{mmol}^{-1} \text{ kg s}^{-1}$)	2.3	± 0.1 (5%)	2.3	± 0.1 (5%)
k_{2mal} (s^{-1})	162	± 25 (15%)	245	± 60 (25%)
k_g ($\text{mmol}^{-1} \text{ kg s}^{-1}$)			0.006	± 0.003 (45%)
k_f ($\text{mmol}^{-1} \text{ kg s}^{-1}$)			0.005	± 0.002 (40%)
k_m ($\text{mmol}^{-1} \text{ kg s}^{-1}$)			0 (lower bound)	-

Figure 2. Postulated Kinetic Mechanisms 1 and 2 with parameter estimates for Model 1 and Model 2 respectively, generated from data from fructose-, glucose- and maltose-dipped potato strips. The rate constants correspond to a temperature of 165 °C.

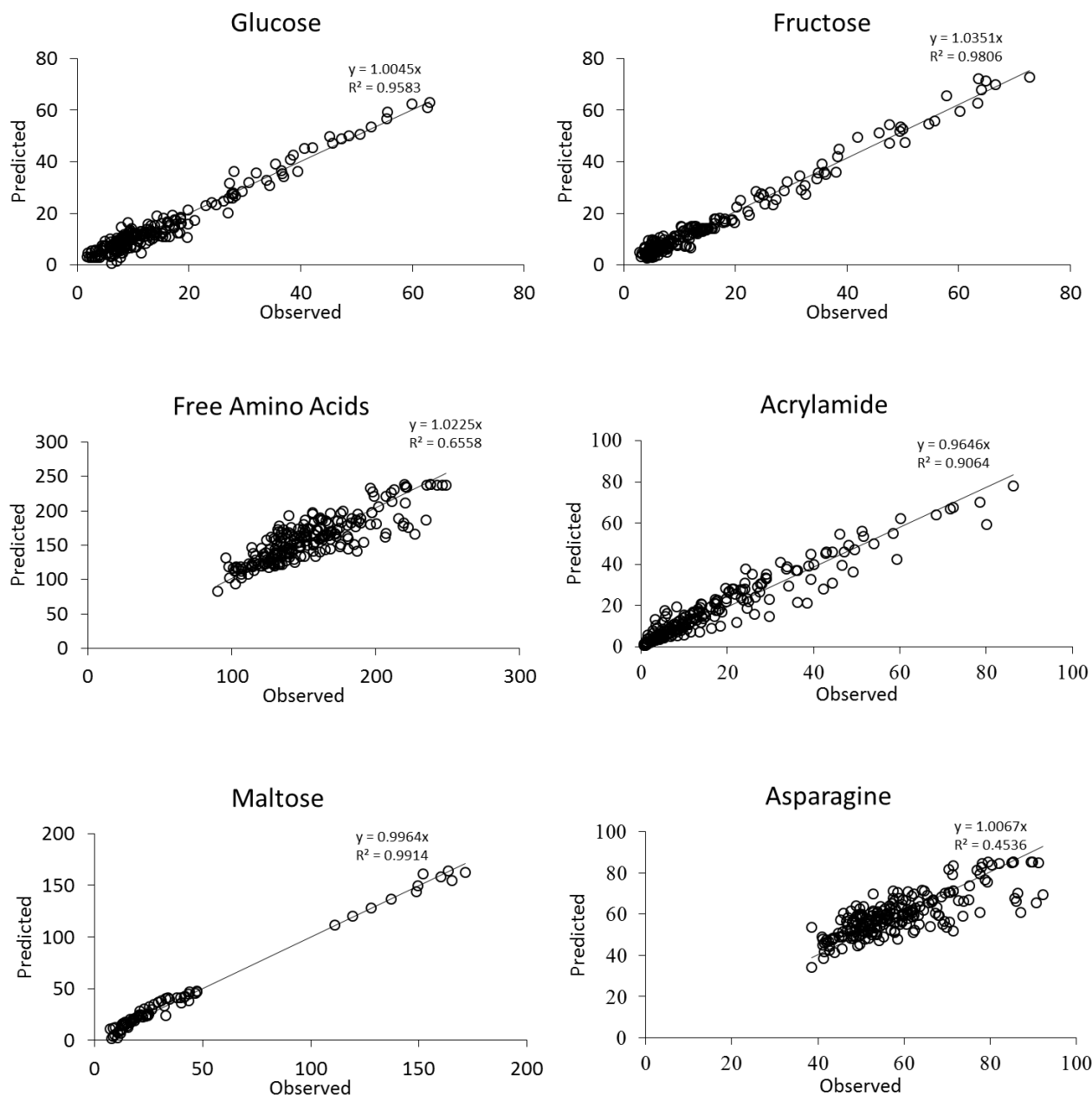


Figure 3. Predicted against observed values for Model 1 for all batches of fries compared with the line of perfect fit ($y = 1$). In all graphs the units are mmol/kg fat-free dry weight, except acrylamide which is expressed as $\mu\text{mol/kg}$ fat-free dry weight. Linear regression was performed on each dataset to produce a slope (y) and R^2 .

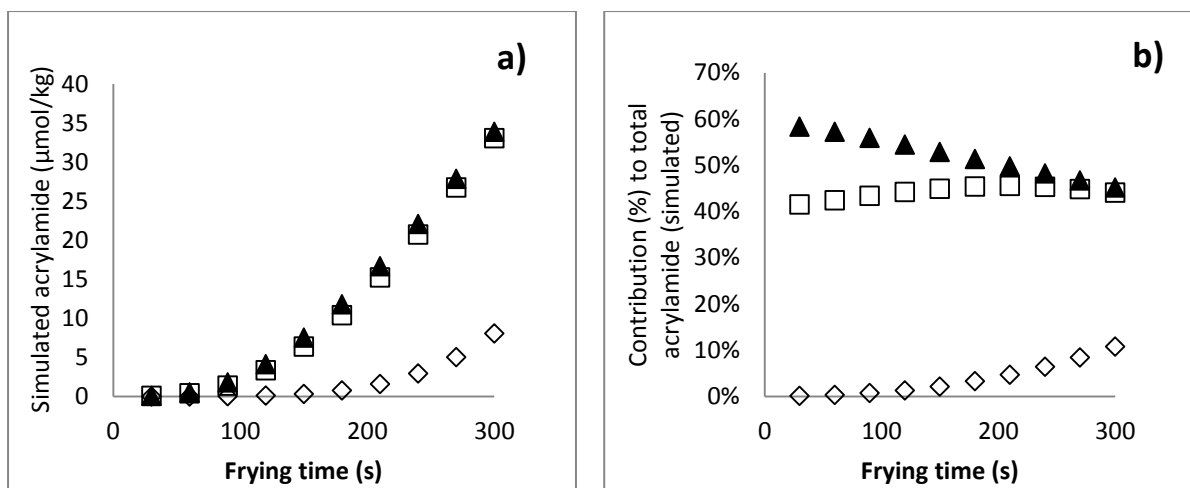


Figure 4. For Kinetic Mechanism 1, comparison of (a) the concentration of acrylamide formed and b) the % contribution to total acrylamide from glucose (□), fructose (▲), and maltose (◇) using model simulations at 165 °C where [FAA] at $t = 0$ was set to 146 mmol/kg dry weight, [Asn] to 60 mmol/kg dry weight, and the sugars were each set at 40 mmol/kg dry weight whilst the other two were set at 0.

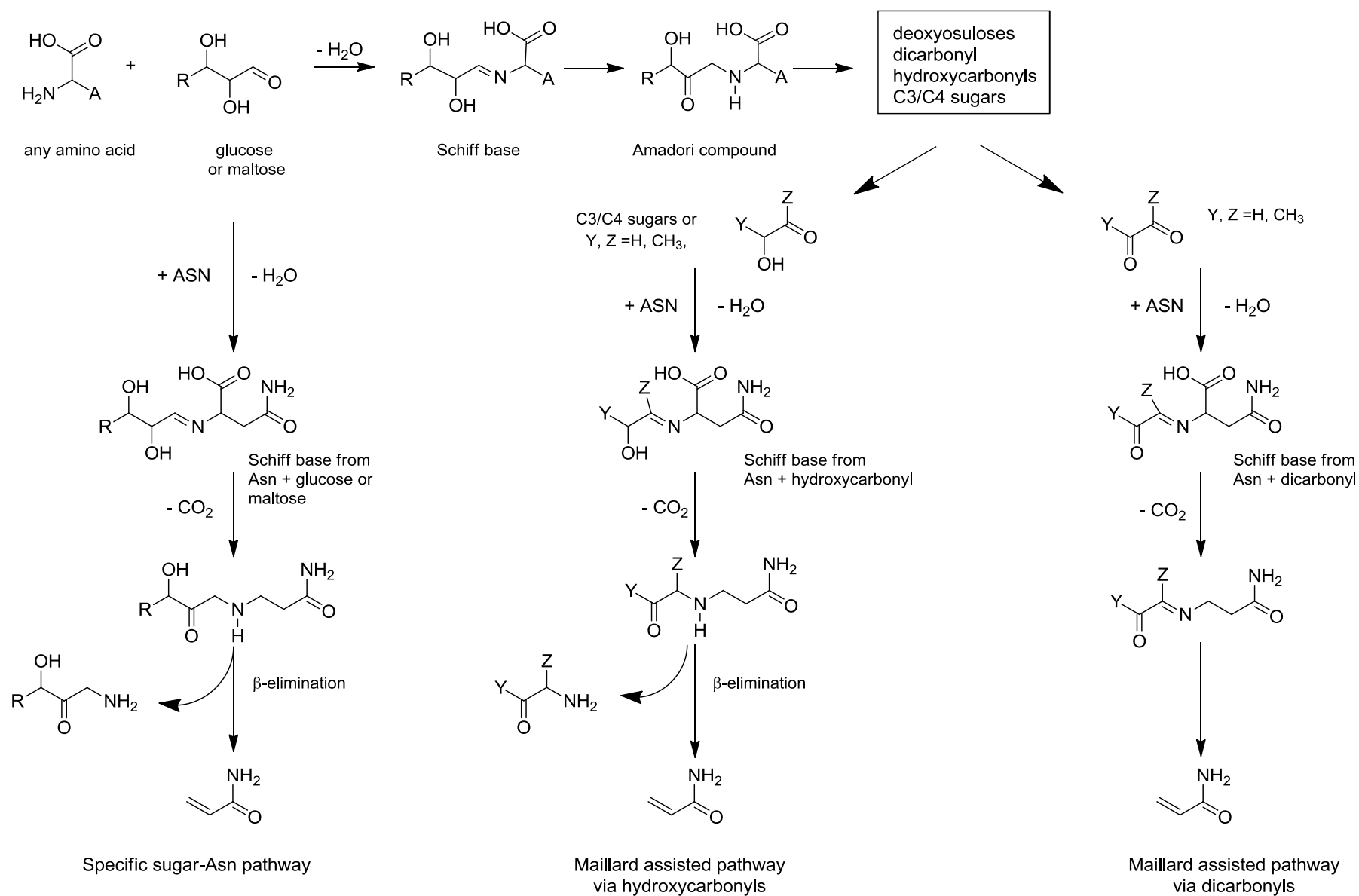


Figure 5. Proposed chemical mechanisms for the formation of acrylamide.

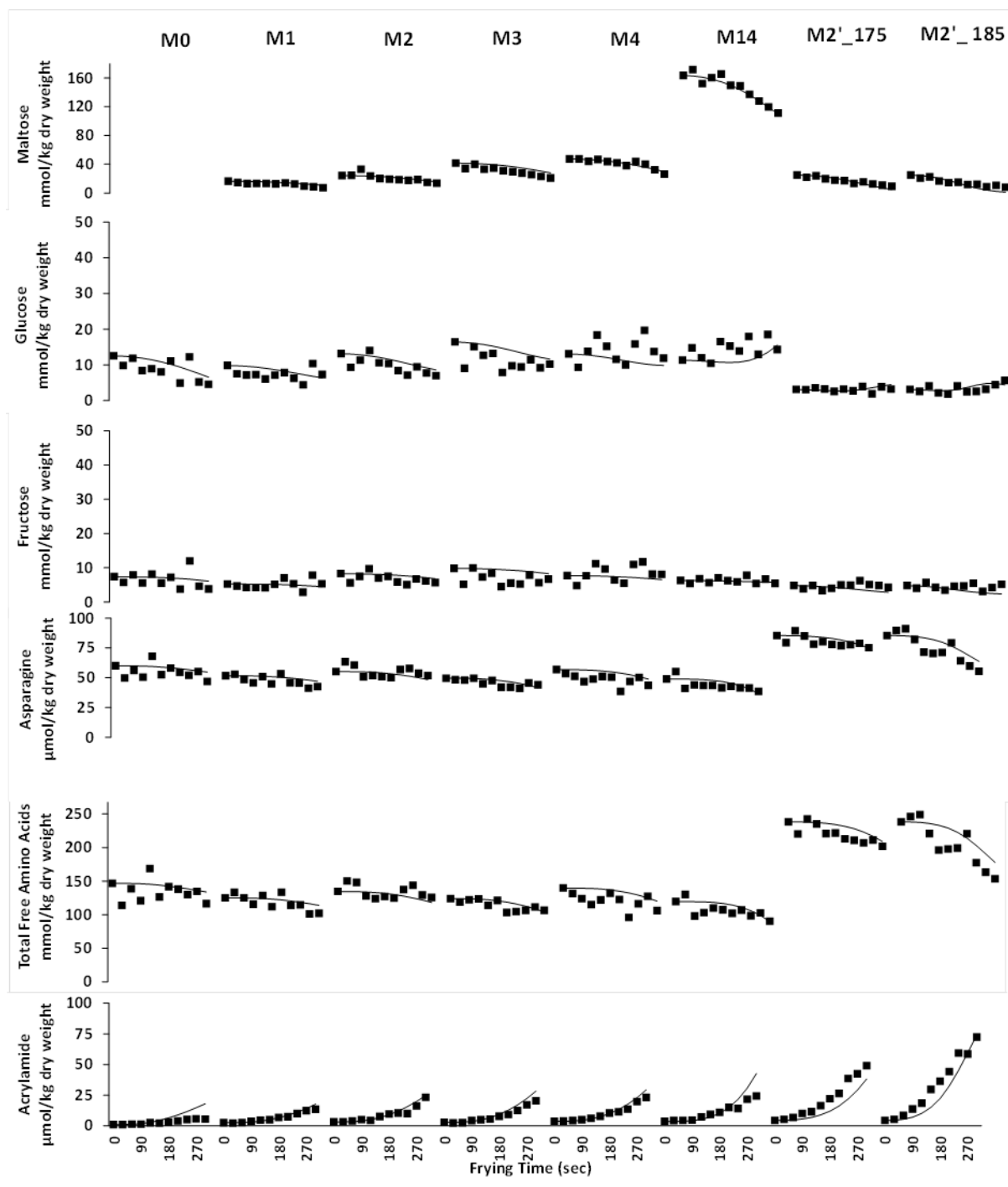


Figure S1 Maltose, glucose, fructose, asparagine, total free amino acids and acrylamide concentrations as a function of time (0–300 s) during finish-frying of eight batches of maltose-dipped potato strips at 165 °C (M0, M1, M2, M3, M4 and M14 represent 0, 1, 2, 3, 4 and 14% dip respectively), 175 °C (M2'_175 = 2.0% dip) and 185 °C (M2'_185= 2.0% dip). Symbols (■) are the experimental data points and the line (—) shows the kinetic Model 1 derived from the combined data set using Kinetic Mechanism 1 (Figure 2).

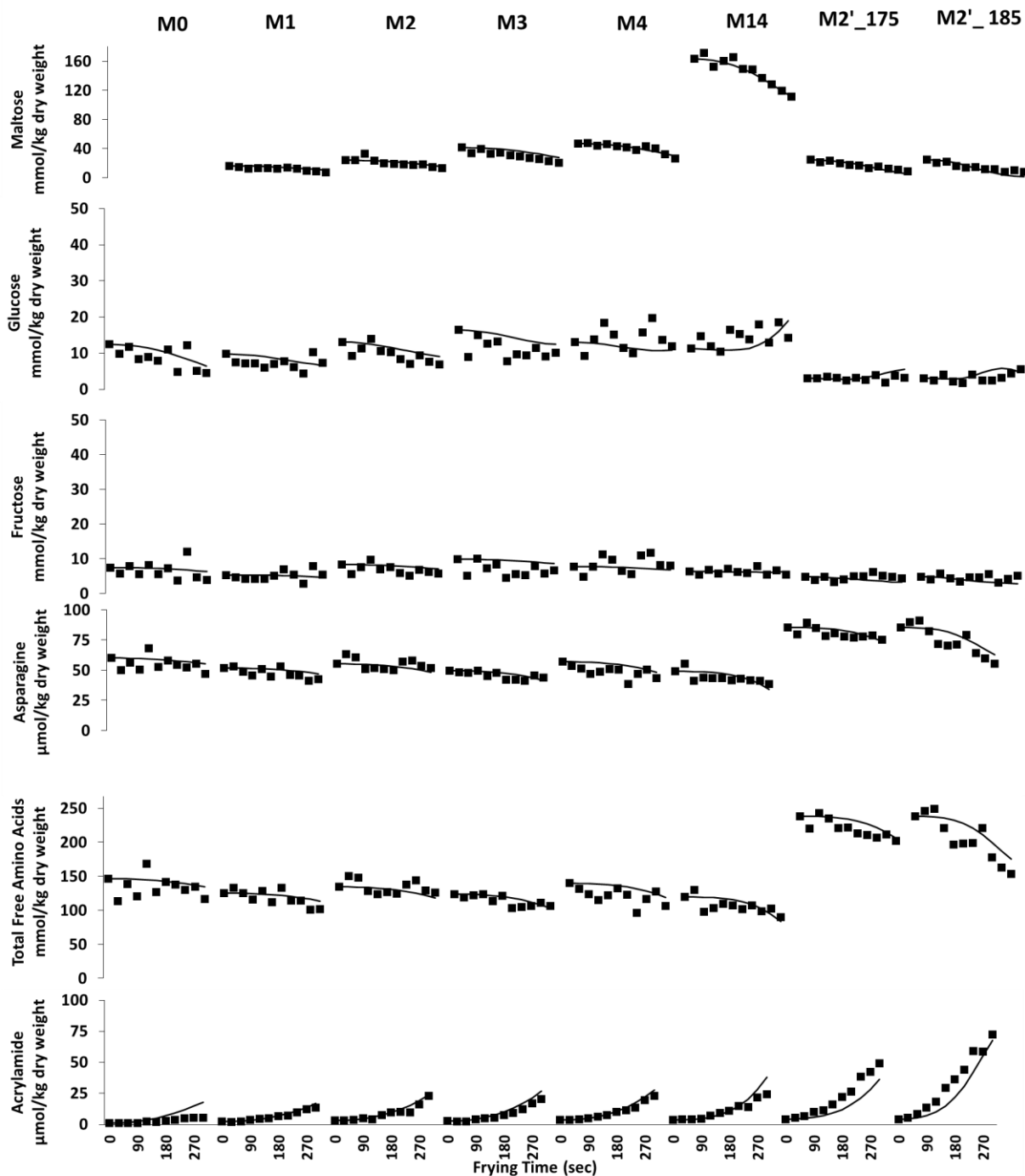


Figure S2. Maltose, glucose, fructose, total amino acids, and acrylamide concentrations as a function of time (0–5 min) during finish-frying of eight batches of maltose-dipped potato strips at 165 °C (M0, M1, M2, M3, M4 and M14 represent 0, 1, 2, 3, 4 and 14% dip respectively), 175 °C (M2'_175 = 2% dip) and 185 °C (M2'_185= 2% dip). Symbols (■) are the experimental data points, and the line (—) shows kinetic model Model 2 derived from the combined data set using Kinetic Mechanism 2 (Figure 2).

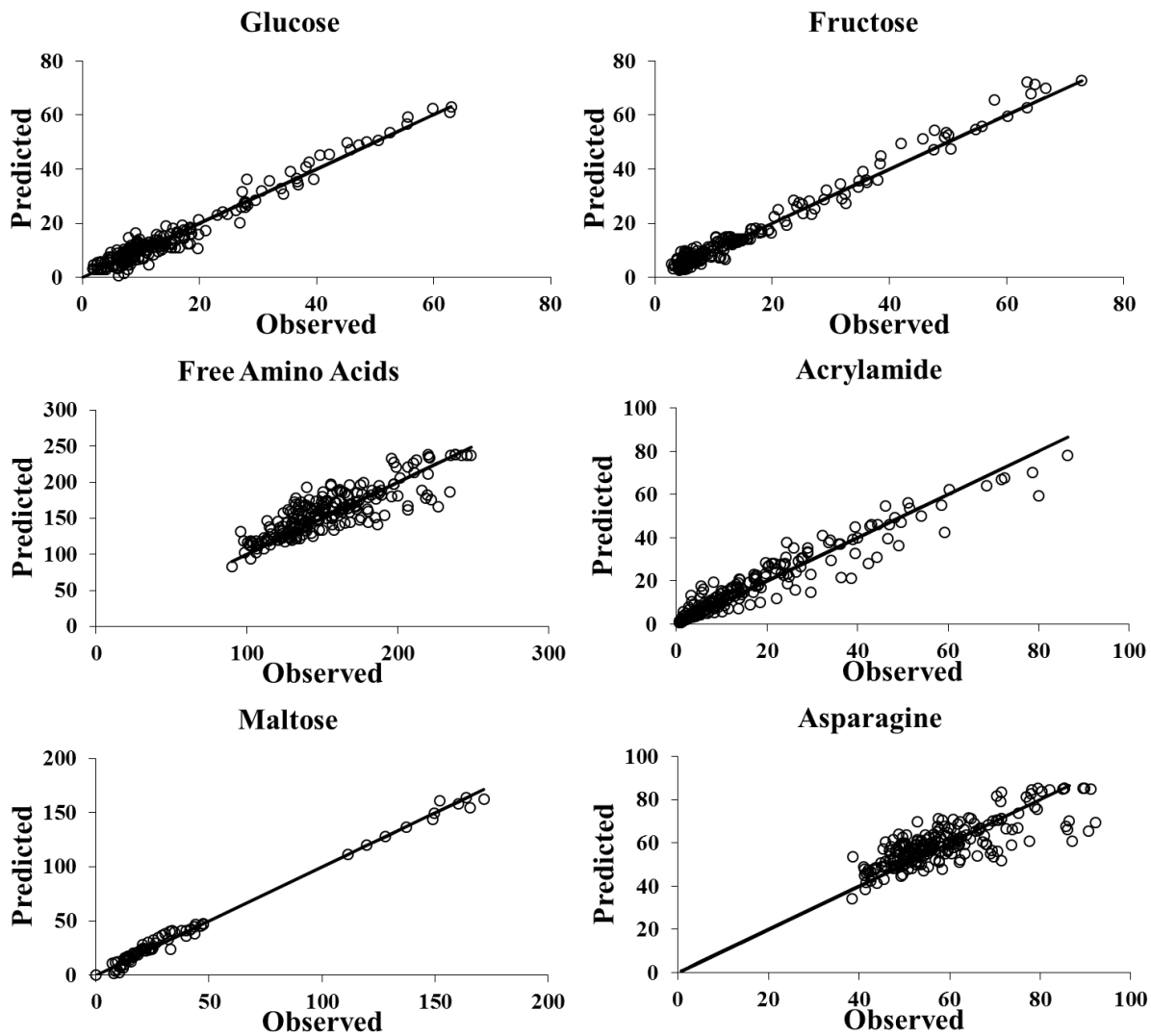


Figure S3. Predicted against observed values for Model 2 for all batches of fries compared with the line of perfect fit ($y = 1$). In all graphs the units are mmol/kg fat-free dry weight, except acrylamide which is expressed as $\mu\text{mol/kg}$ fat-free dry weight.